Nonlinear Optical Interactions in Bacteriorhodopsin Using Z-Scan*

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Nonlinear refractive index coefficient n_2 of bacteriorhodopsin suspensions in water is measured by the Z-scan technique with a low power continuous wave laser at 647.1 manometer wavelength. Our results indicate that both the magnitude and the sign of n_2 depend strongly on the light intensity. Negative values for n_2 are obtained for on axis laser irradiance at the focus above 3 W/cm². The observed self-defocusing phenomena can be attributed to the index change due to the light induced transition between the photochromic states. The results elucidate the origin of n_2 and offer a plausible explanation for the differences in the reported n_2 measurements.

Key words: bacteriorhodopsin, Z-scan, nonlinear, optics

Bacteriorhodopsin (BR) is a photodynamic protein complex isolated from the halophilic bacterium *Halobacterium halobium* found in living systems. The protein is embedded in phospholipid matrix contained in purple patches located within the cell membrane of the bacterium, and acts as an electrochemical shunt by catalyzing photons of light into protons which are used by the cell for energy synthesis. BR has a molecular weight of 26,000 and the chromophore is retinal bound via a Schiff's base to a lysine residue located within the alpha helical structure of the protein. Because of the unique optical and optoelectrical properties of this protein, BR has demonstrated enormous potential for applications[1-6] in optical information processing and storage, ranging from dynamic holography, optical limiting, optical memory, and artificial retina. The biopolymer might be an alternative to conventional materials for future applications in information technologies. Nonlinear optical properties of bacteriorhodopsin have been characterized by several groups. Werner et al.[2] have measured n_2 value using four-wave mixing and self-defocusing. Wang et al.[3] measured n_2 using the Z-scan technique with low power cw lasers. The values of nonlinear refractive index coefficient n_2 measured by different groups differ by about an order of magnitude. In this paper, we report our measurements of the intensity dependence of n_2 by the Z-scan method. The results elucidate the origin of n_2 and offer a plausible explanation for the differences in the reported n_2 measurements.

We used Z-scan to study the nonlinear index of refraction of BR through a wide range of powers at 647.1 nm wavelength, where the absorption is small and the change in refractive index in accordance with the Kramers-Kronig dispersion relations is expected to be large. The BR samples were water suspensions and BR was isolated from wild-type *Halobacterium halobium* at the U.S. Army Natick R.D. & E. Center. The concentration of the samples used is 3.84 mg/ml. The samples had a pH of 7 and the

measurements were obtained at 20°C. The linear absorption coefficient α at 647.1 nm is 3.01 cm⁻¹. We measure the transmittance of the nonlinear medium through a finite aperture in the far field as a function of the sample position Z measured with respect to the focal plane. The effective index of refraction n is expressed as

$$n = n_0 + n_2 I \ , \tag{1}$$

where n_0 is the linear index of refraction, and I is the peak intensity. For a TEM$_{00}$ Gaussian beam traveling in the $+Z$ direction, $\omega^2(z) = \omega_0^2(1 + z^2/z_0^2)$ is the beam radius, $R(z) = (1 + z_0^2/z^2)$ is the radius of curvature of the wave front at z, $z_0 = k\omega_0^2/2$ is the diffraction length of the beam, $k = 2\pi/\lambda$ is the wave vector, and λ is the laser wavelength, all in free space. If the sample length $L < z_0$, the sample is considered thin. Under such an assumption, the amplitude and phase of the electric field as a function of z' are now governed in the slowly varying envelope approximation by a pair of simple equations:

$$\frac{\mathrm{d}\Delta\phi}{\mathrm{d}z'} = \Delta n(I)k \ , \tag{2}$$

$$\frac{\mathrm{d}I}{\mathrm{d}z'} = -\alpha(I)I \ , \tag{3}$$

where z' is the propagation depth in the sample and $\alpha(I)$, in general, includes linear and nonlinear absorption terms. By solving (2) and (3), we can obtain the complex electric field exiting the sample. The far field pattern of the beam at the aperture plane is obtained through the Gaussian decomposition method.

The normalized transmittance $T(z)$ in the far field exhibits a valley-peak for the positive nonlinearity and a peak-valley for the negative one. The peak and valley separation remains nearly constant given by

$$\Delta z_{p-v} \cong 1.7 z_0 \ . \tag{4}$$

The magnitude of the phase distortion can be found either by computer simulation or by using the simple empirical formulas given by Sheik-Bahae et al.[7]

$$\Delta T_{p-v} \cong 0.406(\Delta\phi_0), \quad \text{for} \quad |\Delta\phi_0| \leq \pi \ , \tag{5}$$

for small phase distortion and small aperture. From $\Delta\phi_0$ we can calculate n_2 as

$$n_2 = \frac{\Delta n_0}{I_0} , \qquad (6)$$

where

$$\Delta n_0 = \frac{\Delta\phi_0}{kL_{\text{eff}}} \quad \text{and} \quad L_{\text{eff}} = \frac{(1-e^{-\alpha L})}{\alpha} ,$$

with k the wave vector, L the sample length, α the linear absorption coefficient and I_0 is the on-axis irradiance at the focus which is given by $I_0 = 2P/\pi\omega_0^2$ where P is the power in watts and ω_0 is the radius of the waist at the focus. In our experiments $\omega_0 = 4.5 \times 10^{-3}$ cm. Values of n_2 for the pure sample are obtained from the solution measurements by multiplying them by ρ/C where ρ is the density of BR and C is the solute concentration in g/cc.

Negative values for n_2 in the range -1.0×10^{-4} to -3.0×10^{-3} cm^2/W are obtained for on-axis laser irradiances at the focus in the range 3.7×10^{-2} to 6.0×10^0 W/cm^2. However, for much lower powers we observed unusually large positive n_2 values in the range 6.4×10^{-4} to 4.4×10^{-1} cm^2/W for on-axis laser irradiances at the focus in the range 1.4×10^0 to 2.2×10^{-2} W/cm^2. Figures 1 and 2 show the Z-scan curves at high and low energy, respectively. The change in sign occurs for an irradiance at the focus somewhere between 1.5 and 3 W/cm^2. All the measurements are carried out with the same sample and same experimental set-up. The results obtained for positive and negative values of n_2 as a function of light intensity at the focus are shown in Fig. 3.

This strong dependence of n_2 on the light intensity indicates that the self-defocusing observed is not due to the intrinsic electronic nonlinearity because n_2 value would have been independent of the light intensity in that case. The observed self-defocusing is due to the refractive index change as a result of the photochromic transitions. This change is governed by the Kramers-Kronig dispersion relation that relates the real and imaginary parts of the complex index of refraction to the absorption spectrum. If we denote $A_i(\lambda)$ and $A_f(\lambda)$ for the absorption of the initial

and final states as a function of wavelength, then the corresponding change in refractive index is given by:

$$\Delta n(\lambda) = \frac{2.3026}{2\pi^2 t} \text{P.V.} \int_0^\infty \frac{A_f(\lambda')-A_i(\lambda')}{1-\lambda'^2/\lambda^2} \, d\lambda' , \quad (7)$$

where $\Delta n(\lambda)$ is the change in index of refraction as a function of wavelength, P.V. denotes the Cauchy principal value of the integral, and t is the thickness (nm) of the sample. For bacteriorhodopsin, the most stable intermediate is the M state. It has been shown by Gross *et al.*[8] that the change in refractive index generated by the absorption changes that result from the B to M transition is negative for wavelengths longer than 570 nm in the visible and near infrared. Therefore, we would expect a negative n_2 at 647.1
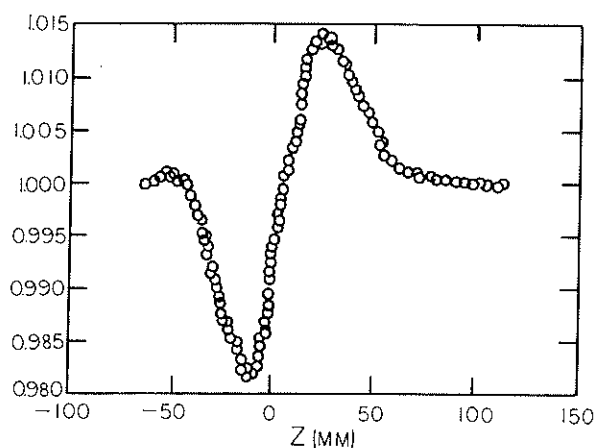

Fig. 2.　Z-scan of BR solution at a laser intensity at the focus of 0.36 W/cm^2 indicating a positive value for n_2.
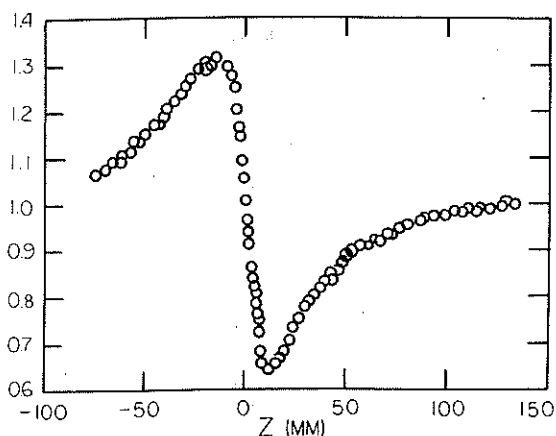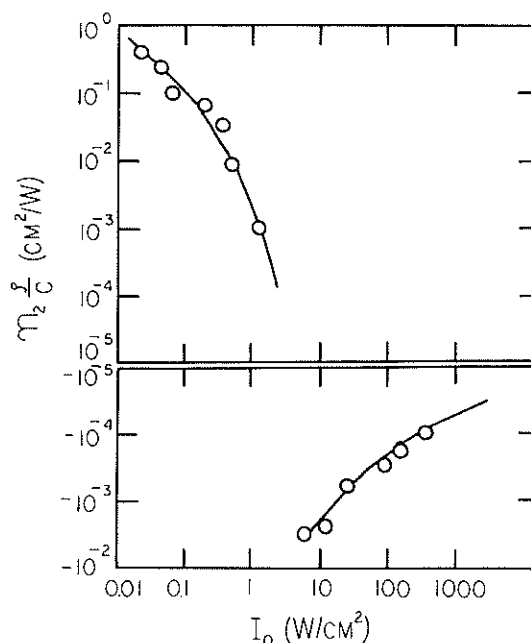

Fig. 3.　Plot of observed positive and negative n_2 values of BR as a function of the on-axis intensity at the focus. Solid line represents the calculated best fit using Eq. (10). The circles are experimental data points.

Fig. 1.　Z-scan of BR solution at a laser intensity at the focus of 160 W/cm^2 indicating a negative value for n_2.

nm. Thus the sign of the n_2 at intensity above 3 W/cm² is consistent with that predicted by the Kramers-Kronig dispersion relation for photochromic transition between B and M state. Effects such as the temperature dependence of the index of refraction governed by the thermal coefficient dn/dt of the refractive index that would result in thermal defocusing can be ruled out. Thermal effects were investigated by Werner *et al.*[2] and found to be negligible around 700 nm wavelength.

The intensity dependence of the n_2 can be explained by the nonlinear saturable absorption. When the BR sample contains a mixture of molecules in i and f states, the refractive index of the material can be written as:

$$n = n_i C_i + n_f C_f , \qquad (8)$$

in steady state condition. Using the two-level rate equation, we have:

$$n = n_i + \frac{\Delta n}{I_s} \cdot \frac{I}{1 + I/I_s} , \qquad (9)$$

where n_i and n_f are the refractive indexes of the initial and final state and C_i and C_f are the ratios of molecules in each state to the total number of molecules, I_s is the saturation intensity and Δn the change in index of refraction given by the Kramer-Kronig relation. From this relation we can derive n_2 as:

$$n_2 = \frac{\Delta n}{I_s} \cdot \frac{I}{1 + I/I_s} . \qquad (10)$$

This equation shows that n_2 is a function of the intensity. When light intensity is much smaller than I_s, $n_2 = \Delta n / I_s$. However, as intensity of the probing light exceeds the saturation intensity, n_2 starts to decrease. The discrepancies in the values previously reported in the literature could be accounted for in terms of the intensity at which the measurements were made. In addition, the equation also shows that n_2 is also strongly dependent on the saturation intensity which is a function of the lifetime of the photochromic state. It has been known that the lifetime of the M state is strongly dependent on pH, humidity, and other environmental factors. These factors could also affect the n_2 value measured. Equation (10) was used to fit the experimental data obtained from the Z-scan measurements. The value of Δn used was 0.0049 which was calculated from the Kramers-Kronig formula (Eq. (7)) for our BR sample under the assumption of 100% photoconversion. The best fit yields a value for the saturation intensity of 2.355 W/cm². The saturation intensity is related to the lifetime of an excited state by $I_s = h\nu / \sigma\tau$ where h is Planck's constant, ν is the photon frequency and σ is the absorption cross section. For BR σ is 2.15×10^{-17} cm².

The value for τ thus obtained is of 6.12×10^{-3} seconds which is consistent with the values for the lifetime of the M state for water solutions of wild-type BR reported in the literature.[9] The solid line in Fig. 3 is the best fit using the above parameters. Our results also agree with the values previously reported[2,3] for n_2 at the intensities that were used in those experiments.

The exact mechanism for the change in sign for the n_2 value at low light intensity is still not clear. The positive n_2 values obtained at very low intensities may be attributed to photochromic transition involving some other intermediates. One possible example is the transition between the dark adapted BR to light adapted BR. The change in the absorption spectrum generated by the dark to light adapted transition would predict a positive n_2 at 647.1 nm.[10] In addition, the saturation intensity for this transition is very low and a large n_2 value is predicted. Further experiments are in progress to elucidate the origin of the positive values of n_2 at very low intensities.

In summary, we have measured third-order nonlinear optical interactions of BR as a function of light intensities using the Z-scan technique. Values of n_2 are obtained for a wide range of light intensities. BR exhibits both positive and negative values of n_2. The change of sign occurs for an on-axis irradiance at the focus in the range 1.5 to 3 W/cm².

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